In short, testis size in F1 hybrids is determined by an interaction between the chromosome complement and the cytoplasm. We are dealing either with a real cytoplasmic effect (determination by the intrinsic properties of the cytoplasm, independent of the chromosomes it carries or carried), or with a maternal effect (determination of the properties of the cytoplasm by those of the chromosomes it carried before fertilization). The data available permit a discrimination between these two possibilities. A backcross male having one half of its autosomes from race A and the other half from race B has small testes irrespective of the source of its cytoplasm. Furthermore, back-cross males having either kind of X-chromosomes and either kind of cytoplasm may have large or small testes depending upon the combination of the autosomes they carry. Hence, in Drosophila pseudoobscura, the difference between the F1 generations of the reciprocal crosses is due to a maternal effect and not to a plasmatic inheritance.

2 Unpublished data of the writer.

THE DETERMINATION OF SEX IN HABROBRACON

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In the parasitic wasp, Habrobracon juglandis (Ashmead), as in other hymenoptera, males are normally haploid, arising from unfertilized eggs. It has been shown by Anna R. Whiting (1925), however, that following inbreeding, males may arise from fertilized eggs. These males are diploid, inheriting a chromosome set from each parent, and are referred to, therefore, as biparental (Torvik 1931). They are commonly sterile. Why some diploid zygotes should give rise to males and others to females has proved a very puzzling problem, and a problem of double interest because of its probable bearing on the normal mechanism of sex determination in the hymenoptera. In other groups of plants and animals a doubling of the whole chromosome complement does not produce changes comparable to the change of sex that it produces in the hymenoptera. Since fertilized and unfertilized eggs have the same chromosomal balance, and, in most respects at least, the same complement of genes, there is no obvious reason why the one should give rise to females and the other to males.
In considerable part, this problem has been solved by recent investigations of Dr. P. W. Whiting and his co-workers. It has been shown that femaleness is caused by heterozygosity for an allelomorphic pair of sex factors, \( X \) and \( Y \), rather closely linked with the fused \((fu)\) locus. The factors \( X \) and \( Y \) may be thought of (Whiting 1933b) as composed of several adjacent or very closely linked genes, some dominant and some recessive. Thus the \( X \) factor or chromosome may contain the genes \( F \) and \( g \), the \( Y \) factor the genes \( f \) and \( G \). Haploid zygotes, carrying either \( X \) or \( Y \) but not both, develop into males, and, in inbred stocks at least, the same is true of homozygous diploid zygotes, \( XX \) or \( YY \). The lines of evidence are two.

First, when black fused males are crossed to orange females heterozygous for fused, pair matings being used, fused females and wild-type biparental males make up either much more than or much less than one-half of all the diploid progeny (Whiting 1933b and Bostian 1935). The results are explained by assuming the parent female to be of the constitution \( FuX/fuY \) and the parent male to be of the constitution \( fuX \) or \( fuY \). If the parent male is \( fuX \), biparental males among the progeny are \( FuX/fuX \) (non-crossovers) and \( fuX/fuX \) (crossovers). If he is \( fuY \), biparental males are \( fuY/fuY \) (non-crossovers) and \( FuY/fuY \) (crossovers). There is about 15% crossing-over between the fused locus and the locus of the sex factor.

Second, the genitalia of right-left mosaic males frequently show feminization, indicating that femaleness results from the interaction between substances produced by two different haploid sets of chromosomes (Whiting, P. W., 1933a; Whiting, P. W., Greb and Speicher, 1934). Mosaic males are recognized by the presence of two types of tissue, one manifesting some given mutant character, the other failing to manifest it. They always come from mothers heterozygous for the given mutant gene, and probably are the result of the simultaneous development of a polar nucleus along with the egg nucleus. If the line of demarcation between the two types of tissue passes near the genitalia, these often show female characteristics. The assumption that one half of the body carries the \( X \) factor, the other the \( Y \) factor and that femaleness is caused by the interaction of diffusible substances produced by these two factors, will adequately explain the observed facts.

While the assumption of a single allelomorphic pair of sex factors (each perhaps a complex of several linked genes) adequately explains the above facts, it leaves one important fact unaccounted for, namely, the relative infrequency with which biparental males appear. If females are \( XY \) and haploid males either \( X \) or \( Y \), half of all biparentals should be \( XX \) or \( YY \) and hence males. As a matter of fact, biparental males never make up as much as 50% of the total of all biparentals. When unrelated wasps are mated, no biparental males are produced, and even in fraternities from related wasps the females are usually at least three or four times as frequent as their biparental brothers.
To meet this difficulty, Whiting (1933b and 1935) has suggested that crosses of unrelated stocks fail to produce biparental males because of heterosyngamy. "Heterosyngamy always occurs when parents are unrelated, for all biparentals from such crosses are females. If parents are related, however, a few biparental males appear because of homoeosyngamy." In other words, X-bearing sperm nuclei tend to unite with Y-bearing egg nuclei, and vice versa, though the strength of the tendency varies with the degree of the relationship of the parents. While this suggestion will explain the low incidence of biparental males, there are a number of reasons for believing that it is not the correct explanation.

Let us first consider the incidence of biparental males in inbred stocks, and see if we find there any evidence of selective syngamy. Matings of orange #3 ♀ ♂ to related type #1 ♂ ♂ gave, in vials a and b when raised at 30°C., 233 type ♀ ♂, 21 type (biparental) ♂ ♂ and 251 orange♂ ♂ (Whiting, P.W., and Anderson, 1932, table 4). Biparental ♂ ♂ thus made up, not 50%, but 7.7% of all biparentals. Experiments carried out by Anna R. Whiting in 1923 (see Whiting, P. W., and Anderson, 1932) showed a high mortality among eggs when the parents were from the same stock. When #3 ♀ ♂ were mated to #3 ♂ ♂, 49.1% of the eggs produced larvae and 81.8% of the larvae produced adults; when #3 ♀ ♂ were mated to unrelated #11 ♂ ♂, 74.3% of the eggs produced larvae and 77.7% of the larvae produced adults. These matings gave 118 ♂ ♂, 115 ♀ ♀; and 41 ♂ ♂, 177 ♀ ♀, respectively, a relative deficiency of biparentals (females) in the case of #3 × #3. Evidently about 43.7% \( \frac{74.3 \times 77.7 - 49.1 \times 81.8}{49.1 \times 81.8} \times 100 \) less of the eggs from related parents are viable than of the eggs from unrelated parents. Moreover, the relative deficiency of biparentals from related parents suggests that the 43.7% is composed largely or entirely of eggs that would develop into biparentals. Let us assume that it is composed entirely of eggs destined, if they survived, to give biparental males, and calculate the expected incidence of biparentals on this basis. Using the data given above for #3 ♀ ♂ × related #1 ♂ ♂, the number of biparental males, on the above assumption, is

\[ 0.437(233 + 21 + 251) + 21 = 242. \]

This is approximately equal to 251, the number of females. Assuming, then, that the excess mortality in inbred stocks as compared with crossbred stocks takes place entirely among potential biparental males, we find that female zygotes and diploid male zygotes are produced with approximately equal frequency. In other words, the sex ratio is

\[ XY:(XX + YY)::1:1. \]

This is the ratio expected in the absence of selective syngamy.

When other data are used, the figures do not always check as well as
they do with the above data, but this is to be expected in view of several important variable factors, particularly the highly variable incidence of haploid males. The two sets of data chosen were selected because they involve essentially the same stocks and the same environmental conditions.

The hypothesis of heterosyngamy thus appears less probable than the hypothesis of differential mortality as an explanation of the observed incidence of biparental males in inbred stocks.

It remains to determine if selective syngamy is the best explanation of the absence of biparental males in fraternities produced by unrelated parents. Certainly there is no evidence for differential mortality in this case, in fact the total mortality among eggs would be insufficient to account for the absence of biparental males if such males were initially formed with the same frequency as females.

The most probable explanation appears to be that, instead of there being a single pair of factors which, when heterozygous, causes diploid zygotes to develop into females, there are a number of such pairs, and that some of these, at least, are on different chromosomes. To take the simplest case (probably much simpler than the actual situation), assume two pairs of factors $X$ and $Y$, and $W$ and $Z$, lying on two different pairs of chromosomes. Females are then $XYWZ$, $XYWW$, $XYYZ$, $XXWZ$ or $YYWZ$. Diploid males are $XXWW$, $XXZZ$, $YYWW$ or $YYZZ$. Crosses between different stocks tend to involve different factors, for example, to be $\varphi XYWW$ times $\sigma YZ$, and hence to give only females in the $F_1$. Of course if only two pairs of factors were involved, individuals from unrelated stocks would not always differ in this fashion; if five or six or more such factors are at work, however, the chance of unrelated parents giving diploid males in the $F_1$ becomes rather slight. Perhaps the most probable assumption is that there are a few major loci, i.e., loci which acting singly, will usually or always cause femaleness when heterozygous, and a much larger number of minor loci, i.e., loci which, acting alone, cannot cause femaleness, but which will cause it in diploid individuals where a number of the loci are heterozygous. There are two lines of evidence in support of this hypothesis.

First, it is in good accord with the known facts of sex determination in other species. It is known that in Drosophila there are multiple sex-determining factors on the $X$-chromosome (Dobzhansky and Schultz, 1934). A closer parallel is found in the fish, Lebistes. In this species, Winge (1932) has shown that there are at least two sex factors located on different chromosomes, and that in some cases one pair, in some cases the other, plays the determining rôle in sex. There is here a precedent for the assumption that the major rôle in sex determination may shift from one chromosome pair to another.

Second, and more important, the assumption of multiple sex-determining
factors in Habrobracon satisfactorily explains the appearance of biparental males following inbreeding. It may be helpful in understanding what follows to note a similarity between our hypothesis and the usual hypotheses explaining hybrid vigor, particularly in their relation to inbreeding. Castle (1934) has written, "It is significant that Whiting reports the production of diploid males only when the uniting gametes are from closely related stock, a condition under which heterosis would be at a minimum."

It is our assumption that the appearance of diploid males following inbreeding is due to the loss of heterozygosity for sex factors, just as the decrease of vigor following inbreeding is due to the loss of heterozygosity for vigor factors or factors in general.

Excellent experimental data on the appearance of diploid males following inbreeding have been published by Bostian (1934). Orange-eyed females were graded up to an inbred stock of b'ack-eyed males, the following system of matings being used:

\[
P_1 \quad 9900 \#3 \times \sigma \sigma \#11
\]

\[
F_1 \quad F_1 990o \times \sigma \sigma O \sigma \sigma \#11
\]

\[
F_2 \quad F_2 990o \times \sigma \sigma O \#11
\]

\[
F_3 \quad F_3 990o \times \sigma \sigma O \sigma \sigma \#11
\]

Etc.

The experiment using these stocks (the 11-9 experiment) was carried on to generation F\textsubscript{17}. In each generation, fraternities segregated rather sharply into those containing biparental males and those not containing biparental males. The percentage of fraternities containing biparental males tended to increase with each generation, the figures being, for the odd generations starting with the F\textsubscript{1}: 38.6, 37.9, 60.7, 77.7, 91.6, 65.7, 88.8, 100. Among fraternities containing biparental males, the percentage of biparental males to all biparentals increased from 9.1 to 17.0 to 25.9 in the F\textsubscript{3}, F\textsubscript{5} and F\textsubscript{7} generations, respectively, and then ran rather constantly near the last figure.

These results are satisfactorily explained by assuming that stock 3 and 11 differ in several non-linked sex factors similar to the X and Y factors of Whiting, females in the two stocks being, for example, \textit{XY-WWMM} and \textit{XXWZLL} (plus possibly several minor factors). Biparentals in the F\textsubscript{1} generation are all heterozygous for one or more of the factors, and hence females. With successive generations of grading up to stock #11, all the sex loci tend to become homozygous except the one for which #11 is itself heterozygous. The rate at which they tend to become homozygous is indicated by the following calculations.

Assume that the parents are

\[
99 \#3 00XYZZ \times \sigma \sigma \#11 OXW \text{ or } OYW
\]
where \(X\) and \(Y\) are one pair of sex factors and \(W\) and \(Z\) another pair of sex factors on another chromosome, such that diploids heterozygous for either or both pairs of sex factors are females. Assume grading up to \#11 males (\(OXW\) or \(OYW\)) through successive generations by the system of matings used by Bostian. The matings are pair matings, i.e., each female mates to but a single male.

Starting with these assumptions, it may be shown that the percentage of matings which will be of types other than \(XYZZ\) or \(YYWZ\) times \(XW\), or \(XYZZ\) or \(XXWZ\) times \(YW\), and hence capable of producing biparental males in the odd numbered generations, will be

\[
\begin{array}{cccc}
F_1 & F_3 & F_5 & F_7 \\
0 & 64\% & 80\% & 83\%
\end{array}
\]

These figures are not in quantitative agreement with Bostian's results in the early generations of the 11-o experiment, but they do fit the later generations, the observed figures, beginning with the \(F_1\) generation being 60.7, 77.7, 91.6, 65.7, 88.8, 100. This may be taken to mean that more than two pairs of sex factors were present in the early generations, but that all except two of them had dropped out by about the seventh generation. In the last generation, the \(Z\) factor also probably dropped out, all females being \(XYWW\), and hence capable of producing biparental males. From the nature of the segregations involved, considerable fluctuation in the proportion of females producing biparental males is expected from generation to generation, so that the exactitude of the agreement between the observed and expected results cannot be used as an argument either for or against our hypothesis. The significant thing is that the hypothesis satisfactorily explains the general trend.

Starting with the same assumptions, it may be shown that the chance that a single female in the \(F_3\) generation will lack the \(Z\) factor is 0.33. In the \(F_5\) generation the chance is 0.49; in the \(F_7\) generation, 0.57. The chance increases slightly with succeeding generations. If the line was propagated by a single female in each odd numbered generation, the \(Z\) factor probably would not be carried more than two or three odd numbered generations, but it might be carried somewhat longer if several females were used in propagation. If three or four pairs of sex loci were involved, the last one, other than the \(X-Y\) locus, might well not become homozygous till the passage of a considerable number of generations. This is in accord with the results obtained by Bostian. In his 11-o experiment, the last sex factor introduced by the \#3 stock and not present in the \#11 stock appears to have been lost in the breeding females of the sixteenth generation, all breeding females of this generation having produced diploid males.

Starting with the same assumptions, and considering only fraternities
where the genetic constitution of the parents is such that biparental males can be produced, it may be shown that the expected per cent of biparentals that will be males (biparental males \times 100/biparentals) is

\[
\begin{array}{cccc}
F_1 & F_2 & F_3 & F_4 \\
0 & 39\% & 41\% & 44\% \\
\end{array}
\]

Bostian's values are much lower than these, but this is to be expected because of the high mortality among diploid males. All that can be said is that the calculated values are of the general order required to fit the facts.

The hypothesis that femaleness in Habrobracon is due to heterozygosity for one or more of a number of sex genes lying in different chromosomes is thus in accord with available data. That such heterozygosity is the only cause concerned in sex determination cannot be asserted. Quite probably the cytoplasm plays a rôle, as suggested by Castle (1934), and some influence of the environment is also probable. The evidence is strong, however, that heterozygosity for multiple sex genes is a principal agent. Further pertinent data may be obtained from either of the two following experiments.

First, linkage tests may reveal other sex factors, in addition to the X-Y factors, borne on other chromosomes.

Second, critical evidence can be obtained by tests of the ratio in which fused and non-fused females appear in the \( F_1 \) of the cross

\[ \Phi \Phi fu/Fu \times \text{unrelated } \Psi \Psi fu. \]

The hypothesis of multiple sex factors predicts a 1:1 ratio of fused to non-fused females. The selective syngamy hypothesis predicts a significant departure from the 1:1 ratio.


THE INHERITANCE AND LINKAGE RELATIONS OF KINKY COAT, A NEW MUTATION IN THE NORWAY RAT

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The present investigation is concerned with a study of the inheritance and linkage relations of kinky coat, a new recessive mutation in the Norway rat. This mutation was discovered by Dr. H. W. Feldman in a stock of Norway rats at the University of Michigan and he kindly turned it over to me for a study of its linkage relations. Since this character has not to my knowledge been described in the literature, I shall give a brief description of it. The credit for its discovery, however, belongs to Dr. Feldman.

The appearance of rats homozygous for this character is quite striking, as every hair is kinky giving the coat a distinctly curly appearance. New-born rats that will later have a kinky coat can be distinguished from their normal sibs by their curly vibrissae.

Kinky is the second mutation of this kind to have been observed in the Norway rat, Dr. Helen Dean King (1932) having already described a dominant “curly coat” character which arose in a strain of captive gray Norway rats.

Similar mutations affecting hair structure have been found in other rodents. Castle and Nachtsheim (1933) have described three genetic types of “rex” in the rabbit, all of which are recessive and indistinguishable in appearance. As in the kinky rats, rabbits homozygous for rex can be distinguished at birth by their curly vibrissae. The coat however is plush-like rather than kinky or curly, the guard hairs being largely lacking and the other hairs shortened. The genes for two of these rex mutations lie in the same chromosome. Crew (1933) has reported a recessive “waved” coat character in the house mouse (Mus musculus). Mice homozygous for this mutation have a distinctly curly coat when about two weeks of age. Later however, the curliness disappears and it is difficult to distinguish adult waved mice from normals.

Feldman (unpublished data) found that the new kinky coat mutation in the Norway rat behaved as a simple recessive Mendelian character. My own results, which substantiate his conclusions, are shown in table 1.